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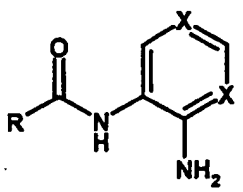
(54) Title: N-MONOACYLATED DERIVATIVES OF O-PHENYLENEDIAMINES, THEIR SIX MEMBERED HETEROCYCLIC ANALOGUES AND THEIR USE AS PHARMACEUTICAL AGENTS



(I)

(57) Abstract: Compounds of formula (I), in which X independently from each other denotes a CH group or a nitrogen atom and R represents an optionally substituted five or six membered nonaromatic carbocyclic ring or an nonaromatic or aromatic heterocyclic ring, whereby the ring system may also be condensed with a 6-membered, optionally substituted carbocyclic aromatic ring; and its pharmaceutically acceptable salts; as pharmaceuticals for the treatment of cancer.

N-Monoacylated derivatives of o-phenylenediamines, their six membered heterocyclic analogues and their use as pharmaceutical agents

This invention relates to antitumor agents and, in particular, to the use of monoacylated, aromatic, o-diamine substituted aromatic or heteroaromatic six membered ring systems or pharmaceutically-acceptable salts thereof. Based on their antiproliferative and differentiation-inducing activity, which results in inhibition of tumor cell proliferation, induction of apoptosis and inhibition of invasion, these compounds are useful for the treatment of diseases such as cancer in humans or animals.

Cancer is one of the major causes of death, exceeding heart and cerebrovascular diseases, and so many studies have been conducted with enormous expense and time to overcome cancer. However, in spite of a variety of therapies such as surgical operation, radiation therapy and chemotherapy, there is still a great need for improved anticancer therapeutics. Among these therapies, chemotherapy is one of the main areas for cancer treatment. Most drugs show their effect by affecting mainly DNA to express their cytotoxicity and then, in consequence injuring tumor cells. However, lacking selectivity, they do not sufficiently differentiate between tumor cells and normal cells, and therefore, adverse reactions expressed in normal cells have limited their use in therapy. Up to now, no satisfactory drugs have been discovered, and thus an anticancer drug with reduced toxicity, better tolerability and a high therapeutic effect is very much desired.

Meanwhile, differentiation-inducing, antiproliferative agents among anticancer drugs are intended to induce differentiation of tumor cells for controlling their infinite proliferation and survival, rather than directly killing the cells.

The agents may therefore be inferior to the anticancer drugs directly killing tumor cells, with regard to involution of a tumor, but may be expected to have reduced toxicity and different selectivity and are expected to exert additive effects in combination with other antitumor therapeutics or treatments.

Preferred embodiments of this invention provide compounds which exhibit differentiation-inducing and antiproliferative effects and therefore are useful as

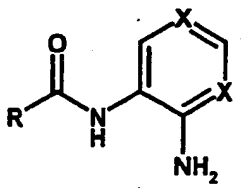
pharmaceutical agents for treatment of malignant tumors, autoimmune diseases, dermatologic diseases and diseases caused by parasites.

The invention relates to new mono-N-acylated o-diamino substituted aromatic or heteroaromatic six membered ring systems and its pharmaceutically acceptable salts, which inhibit cell-proliferation activity and are therefore useful for the treatment of diseases such as cancer in humans or animals. The invention also relates to processes for the manufacturing of these o-diamine derivatives, to pharmaceutical compositions containing them and to their use in the manufacture of drugs for the treatment of diseases like cancer.

EP-A 0 847 992 describes monoacylated o-phenylendiamine derivatives as cell differentiation inducers. The same type of compounds is also the subject of EP-A 0 242 851. The compounds described in these applications are almost exclusively o-phenylene derivatives monoacylated with derivatives of benzoic acid. However, there is still a need to provide compounds with improved properties such as increased tolerability, less toxicity and less side effects.

Monoacylated o-phenylendiamines are known in the art as precursors for the preparation of the corresponding benzimidazoles, such preparation methods are e.g. described in DE-A 2 062 265; FR 2 167 954; Rastogi, R., and Sharma, S., Indian J. Chem., Sect. B, 21B (5) (1982) 485-487; Moll, R., et al., Z. Chem. 17 (1977) 133-134; and Hassan, H., et al., Indian J. Chem. 39B (2000) 764-768.

The present invention concerns new compounds of the following general formula (I)



(I)

in which

X independently from each other denotes a CH group or a nitrogen atom and

R represents an optionally substituted five or six membered nonaromatic carbocyclic ring or an nonaromatic or aromatic heterocyclic ring, whereby the ring system may also be condensed with a 6-membered, optionally substituted carbocyclic aromatic ring;

with the proviso that

(a) if X denotes CH, R does not represent

halogenated benzothiophene; halogenated thiazolyl; N-benzyl-2-acetylamino-4,5-dimethylpyrrole-3-yl; optionally substituted 1,4-oxathiine-3-yl; optionally substituted pyridinyl; pyridazine-5-yl which is substituted by one to three substituents selected from methyl, methoxy, methoxycarbonyl or carboxyl;

or

(b) if one or both X in formula (I) denote NH, R does not represent optionally substituted pyridinyl.

The present invention also encompasses pharmaceutically acceptable salts or prodrugs of the compounds of formula I as well as the use of these compounds to produce pharmaceutical agents.

The symbol X containing ring systems are phenyl, pyridine or pyrimidine whereby the phenyl ring may be substituted by one or two halogen atoms, preferably by chlorine.

The non-aromatic carbocyclic ring system represented by R in the general formula (I) is understood as a carbocyclic ring which may be constructed from single and double bonds to form a five or six membered ring. The ring system must contain at least one single sp3 hybridized carbon atom. Examples include cyclopentane, cyclohexane, cylohexene, cyclohexadiene, preferred is cyclohexene. The nonaromatic or aromatic heterocyclic ring in the substituent R in formula (I) may be a five or six membered, saturated or unsaturated ring containing 1 to 4 heteroatoms selected from nitrogen, oxygen or sulfur. This heterocyclic ring includes, for example, piperazine, piperidine, morpholine, pyrrolidine, pyrimidine, pyrazine, pyridazine, tetrahydro-pyridazine, pyrrole, furane, imidazole, pyrazole, dihydro-pyrazole, triazole, thiophene, thiazole, oxazole, isothiazole, isoxazole, dihydro-isoxazole, thiadiazole, tetrazole whereby the heterocyclic rings pyrrolidine,

dihydropyrazole and tetrahydro-pyridazine may carry an oxo-group. Preferred rings are thiophene, furane, pyrazole, imidazole, isothiazole, isoxazole, triazole.

The above mentioned heterocycles may be fused with a 6 membered, optionally substituted carbocyclic aromatic ring, examples are benzothiophene, benzofurane, indole, benzimidazole, indazole, benzothiazole, benzoxazole, quinoline, isoquinoline, benzoindole; preferred are benzofurane, benzothiophene, indole.

The ring system R or its condensed derivatives may be substituted with one or more substituents selected from aralkyl, aryl, hetaryl, hetaralkyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkinyl, C_1-C_7 acyl, C_1-C_6 alkyloxy, C_2-C_6 alkenyloxy, C_1-C_6 alkylamino, di(C1-C_6)alkylamino, amino, hydroxy, halogen, nitro, carboxyl, carboxamido, aminocarbonyl or trifluoromethyl groups.

Aryl, hetaryl, aralkyl and hetaralkyl substituents which may be substituents of the ring R in the general formula (I) may itself be substituted by one or more substituents selected from C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkinyl, C_1-C_7 acyl, C_1-C_6 alkyloxy, C_1-C_6 alkylamino, C_1-C_6 dialkylamino, amino hydroxy, halogen, nitro, carboxyl, carboxamido, aminocarbonyl, trifluoromethyl groups.

An aryl group represents a carbocyclic conjugated ring system, for example phenyl, naphthyl, preferably phenyl, which may be unsubstituted or substituted with the above-mentioned substituents.

An aralkyl group denotes the combination of an aryl group described above with a C_1-C_6 alkyl group connected through the alkyl part. Examples are benzyl, phenethyl and naphthylmethyl, preferred is benzyl and phenethyl wherein the phenyl ring may be substituted by the above-mentioned substituents.

Hetaryl groups which may be substituents of the ring R represent a mono- or bicyclic conjugated ring system containing 1 to 4 heteroatoms selected from nitrogen, oxygen or sulfur and carbon atoms to finish the ring system. Such rings include for example piperazine, piperidine, morpholine, pyrrolidine, pyridine, pyrimidine, pyrazine, pyridazine, pyrrol, furane, imidazole, pyrazole, triazole, thiophene, thiazole, isothiazole, isoxazole, thiadiazole, tetrazole, oxazole, preferred

are thiophene, furane, pyrazole, imidazole, isothiazole, isoxazole, triazole pyridine, pyrimidine, pyrazine, benzofurane, indole, benzothiophene, quinoline.

A hetaralkyl group denotes the combination of a hetaryl group described above with a C_1-C_6 alkyl group connected through the alkyl part to the residue, preferred are pyridylmethyl, thienylmethyl, imidazolylmethyl.

C_1-C_6 alkyl residues as such or in combinations with other residues denote preferably methyl, ethyl, propyl, isopropyl or tert.-butyl.

C_2-C_6 alkenyl denotes preferably allyl or pentadienyl. C_2-C_6 alkinyl denotes preferably propargyl.

C_2-C_6 alkenyloxy denotes preferably allyloxy.

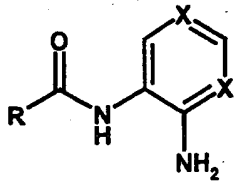
C_1-C_7 acyl denotes -C(O)-C_1-C_6-alkyl or -C(O)H, preferably an acetyl group.

The alkyl residues can optionally be interrupted once or several times by heteroatoms (O, S, N) to form e.g. CH_3-O-CH_2-CH_2-O residue.

Halogen is understood as fluorine, chlorine, bromine, iodine, preferred fluorine or chlorine.

Preferred compounds are compounds of formula I wherein X is CH.

A further embodiment of the invention are pharmaceutical compositions containing as an active ingredient a compound of formula I



(I)

in which

X independently from each other denotes a CH group or a nitrogen atom and

R represents an optionally substituted five or six membered nonaromatic carbocyclic ring or an nonaromatic or aromatic heterocyclic ring, whereby the ring system may also be condensed with a 6-membered, optionally substituted carbocyclic aromatic ring;

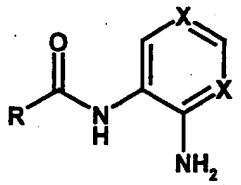
and its pharmaceutically acceptable salts,

with the proviso, that

R does not represent optionally substituted pyridinyl.

Preferred pharmaceutical compositions are compositions containing a compound of formula I wherein X is CH and R is an unsubstituted or substituted benzofurane, benzothiophene, indole cyclohexene, pyrazole, thiazole, pyrrole, isoxazole or furane ring.

Another embodiment of the invention is the use of the compounds of formula I



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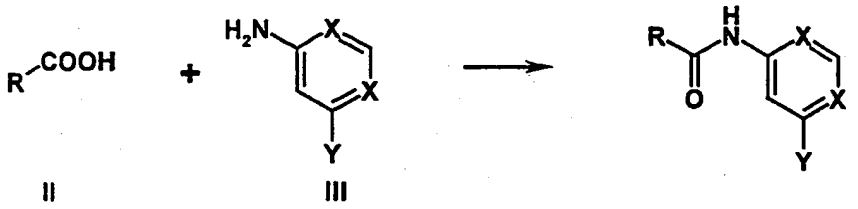
and its pharmaceutically acceptable salts
with the proviso, that R does not represent optionally substituted pyridinyl,
as medicaments for the treatment of cancer.

The compounds of the general formula (I) can be prepared according to well known processes. A carboxylic acid with the general formula (II) in which the residues R has the meaning described above is reacted with a compound with the

general formula (III) in which the residue Y represents an amino or a protected amino group and X represents carbon or nitrogen and if X denotes carbon the six membered ring may be substituted by one or two halogen atoms.



II III

To accomplish this amide bond formation the carboxylic acid is activated using methods known from peptide chemistry. Such activation reagents comprise the formation of mixed anhydrides using chloroformates, activation with carbodiimides, N,N'-carbonyldiimidazol, uroniumsalts, e.g., O-(1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoro-borate, phosphorus based reagents e.g. bis-(2-oxo-3-oxazolidinyl)-phosphinic acid chloride. Carboxylic acids may also be converted to their acid chlorides using known methods e.g. by treatment with thionylchloride or oxalic acid dichloride. Y represents an amino group and this second amino group may be protected to avoid bis-condensation of the carboxylic acid. The protection groups are cleaved after the formation of the amide bond. Protection groups for the amino group are known from peptide chemistry,. e.g. benzyloxycarbonyl (cleavage by hydrogenation or hydrobromic acid in acetic acid), t-butoxycarbonyl (cleavage by strong acids e.g. trifluoroacetic acid), 9-fluorenmethoxycarbonyl (cleavage by secondary amines e.g. piperidine). The nitro group can also be used as a precursor for the amino group. In this case it is best to use a strongly activated carboxylic acid for the acylation of the weakly basic amino group, preferably the acid chloride. The amino group is generated through reduction of the nitro group either by catalytic hydrogenation e.g. over palladium on carbon or by treatment with reducing agents e.g. zinc in acetic acid, tin(II)chloride or sodium dithionite/$NaHCO_3$. It is also possible not to use a protecting group. In this case it is best to use an excess of acid to achieve a complete acylation of the primary amino groups. During work-up the desired product has to be separated from the product resulting from the disubstitution. This is easily accomplished by exploiting the basic properties of the product using an ion

exchanger, preferably a macroporous type ion exchanger which can also be used in organic solvents.

The carboxylic acids and the aromatic amines are commercially available, described in the literature or can be prepared analogously to published methods.

Compounds of the general formula I can contain one or several chiral centres and can then be present in a racemic or in an optically active form. The racemates can be separated according to known methods into the enantiomers. Preferably diastereomeric salts which can be separated by crystallization are formed from the racemic mixtures by reaction with an optically active acid such as e.g. D- or L-tartaric acid, mandelic acid, malic acid, lactic acid or camphorsulfonic acid.

The compounds of the present invention may exist as salts of organic or inorganic acids. Salts such as acetates, citrates or hydrochlorides are mainly used as pharmaceutically acceptable materials which are produced in the usual manner e.g. by titrating the compounds with or organic or inorganic acids selected from acetic acid, citric acid or hydrochloric acid. The salts are usually purified by reprecipitation from water/acetone.

The new compounds of formula I and salts thereof according to the invention can be administered enterally or parenterally in a liquid or solid form. In this connection all the usual forms of administration come into consideration such as for example tablets, capsules, coated tablets, syrups, solutions, suspension etc. Water which contains additives such as stabilizers, solubilizers and buffers that are usual in injection solutions is preferably used as the injection medium.

Such additives are e.g. tartrate and citrate buffer, ethanol, complexing agents (such as ethylenediaminetetraacetic acid and non-toxic salts thereof), high-molecular polymers (such as liquid polyethylene glycols) to regulate viscosity. Liquid carrier substances for injection solutions have to be sterile and are preferably dispensed into ampoules. Solid carrier substances are e.g. starch, lactose, mannitol, methylcellulose, talcum, highly dispersed silicic acids, higher molecular fatty acids (such as stearic acid), gelatins, agar-agar, calcium phosphate, magnesium stearate, animal and vegetable fats, solid high-molecular polymers (such as polyethylene

glycols); suitable preparations for oral application can optionally also contain flavourings and sweeteners.

The dosage depends on various factors such as manner of administration, species, age and/or individual state of health. The doses to be administered daily are about 5-400 mg/kg, preferably 10-100 mg/kg and can be taken singly or distributed over several administrations.

The invention is further illustrated by the following, non-limiting examples.

Example 1

The compounds listed in Table 1 have been prepared according to the following general procedure:

The carboxylic acid (appr. 20 mg, 1.2 equivalents) was dissolved in a 0.2 M solution of diisopropylamine in dimethylformamide corresponding to 3.6 equivalents of base. To the stirring solution was added a 0.1 M solution of O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) in DMF corresponding to 1.20 equivalents, followed after 60 min by a 0.1 M solution of the diamine Aryl(NH_2)$_2$ in DMF corresponding to 1 equivalent. The mixture was stirred overnight. The product was isolated by method A or method B.

Method A: Acidic Ion exchange column

The crude reaction mixture was loaded onto an acidic ion-exchange column (45 mm, 15 mm ID) charged with Fractogel EMD SO_3- 650 (S) previously conditioned with 1 molar sulphuric acid in methanol/water (9:1)and washed with methanol/water 9:1. The column was rinsed with water followed by methanol. The product was then eluted with 0.4 M pyridine in methanol followed by 0.2 M ammonia in methanol. The product fractions were identified by LC/MS, combined and evaporated.

Method B:

The crude reaction mixture was evaporated, the residue dissolved in methanol and loaded onto a reversed-phase column (Kromasil C18,10 µm, 250 mm, 20 mm ID). The column was eluted with a gradient of methanol in water (50 % methanol/water - 100 % methanol).The product fractions were identified by LC/MS, combined and evaporated.

Product identity and purity were confirmed by mass specrometry (atmosperic pressure ionization) and HPLC equipped with mass detection (LC/MS).

Table 1

Number	MolName	Work-up procedure	MW found [M+H]	Exact MW [M+H] calc'd
1	Benzofuran-2-carboxylic acid (2-amino-phenyl)-amide	A	253.3	253.10
2	Furan-2-carboxylic acid (2-amino-phenyl)-amide	A	203.3	203.08
3	1-Methyl-1H-pyrrole-2-carboxylic acid (2-amino-phenyl)-amide	A	216.2	216.11
4	3,5-Dimethyl-isoxazole-4-carboxylic acid (2-amino-phenyl)-amide	A	232.3	232.11
5	5-Oxo-pyrrolidine-2-carboxylic acid (2-amino-phenyl)-amide	A	220.3	220.11
6	1H-Pyrrole-2-carboxylic acid (2-amino-phenyl)-amide	A	202.3	202.10
7	5-Methyl-3-phenyl-isoxazole-4-carboxylic acid (2-amino-phenyl)-amide	A	294.3	294.12
8	5-Phenethyl-4,5-dihydro-isoxazole-3-carboxylic acid (2-amino-phenyl)-amide	A	310.4	310.16
9	5,6-Dimethoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-phenyl)-amide	A	329.5	329.10
10	3-Methyl-isoxazole-5-carboxylic acid (2-amino-phenyl)-amide	A	218.3	218.09

Number	MolName	Work-up procedure	MW found [M+H]	Exact MW [M+H] calc'd
11	2,5-Dimethyl-2H-pyrazole-3-carboxylic acid (2-amino-phenyl)-amide	A	231.3	231.12
12	1,5-Dimethyl-1H-pyrazole-3-carboxylic acid (2-amino-phenyl)-amide	A	231.3	231.12
13	5-Propoxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	310.4	310.16
14	5-Allyloxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	308.5	308.14
15	3H-Benzo[e]indole-2-carboxylic acid (2-amino-phenyl)-amide	A	302.5	302.13
16	5-Methyl-2H-pyrazole-3-carboxylic acid (2-amino-phenyl)-amide	A	217.3	217.11
17	3-Phenyl-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	328.4	328.15
18	3-Methyl-5-phenyl-isoxazole-4-carboxylic acid (2-amino-phenyl)-amide	A	294.3	294.12
19	1-Acetyl-piperidine-4-carboxylic acid (2-amino-phenyl)-amide	A	262.4	262.16
20	Cyclohex-1-enecarboxylic acid (2-amino-phenyl)-amide	A	217.3	217.13
21	3-(2-Methoxy-ethoxy)-thiophene-2-carboxylic acid (2-amino-phenyl)-amide	A	293.4	293.10
22	1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carboxylic acid (2-amino-phenyl)-amide	A	323.5	323.15
23	7-Methoxy-benzofuran-2-carboxylic acid (2-amino-phenyl)-amide	A	283.3	283.11
24	1H-Imidazole-2-carboxylic acid (2-amino-phenyl)-amide	A	203.3	203.09
25	5-(4-Chloro-phenyl)-1H-pyrazole-3-carboxylic acid (2-amino-phenyl)-amide	A	313.3	313.09
26	7-Methoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-phenyl)-amide	A	299.4	299.09

Number	MolName	Work-up procedure	MW found [M+H]	Exact MW [M+H] calc'd
27	2-Phenyl-2H-[1,2,3]triazole-4-carboxylic acid (2-amino-phenyl)-amide	A	280.3	280.12
28	2-Chloro-thiazole-4-carboxylic acid (2-amino-phenyl)-amide	A	254.3	254.02
29	2-Benzyl-5-methyl-2H-pyrazole-3-carboxylic acid (2-amino-phenyl)-amide	A	307.4	307.16
30	5-Methyl-1-phenyl-1H-pyrazole-3-carboxylic acid (2-amino-phenyl)-amide	A	293.5	293.14
31	5-Methyl-1-phenyl-1H-[1,2,3]triazole-4-carboxylic acid (2-amino-phenyl)-amide	A	294.3	294.14
32	5-Methyl-2-phenyl-oxazole-4-carboxylic acid (2-amino-phenyl)-amide	A	294.3	294.12
33	2,5-Dimethyl-4-nitro-2H-pyrazole-3-carboxylic acid (2-amino-phenyl)-amide	A	276.3	276.11
34	1H-Indole-2-carboxylic acid (2-amino-phenyl)-amide	A	252.3	252.11
35	4-Acetyl-3,5-dimethyl-1H-pyrrole-2-carboxylic acid (2-amino-phenyl)-amide	A	272.4	272.14
36	6-Oxo-1,4,5,6-tetrahydro-pyridazine-3-carboxylic acid (2-amino-phenyl)-amide	A	233.4	233.10
37	5-Phenyl-isoxazole-3-carboxylic acid (2-amino-phenyl)-amide	A	280.3	280.11
38	Thiazole-4-carboxylic acid (2-amino-phenyl)-amide	A	220.2	220.05
39	Benzofuran-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	321.2	321.02
40	Furan-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	271.2	271.00
41	3,5-Dimethyl-isoxazole-4-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	300.2	300.03
42	5-Oxo-pyrrolidine-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	288.2	288.03

Number	MolName	Work-up procedure	MW found [M+H]	Exact MW [M+H] calc'd
43	5-Methyl-3-phenyl-isoxazole-4-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	362.4	362.05
44	5,6-Dimethoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	397.3	397.02
45	2,5-Dimethyl-2H-pyrazole-3-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	299.4	299.05
46	1,5-Dimethyl-1H-pyrazole-3-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	299.4	299.05
47	5-Propoxy-1H-indole-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	378.3	378.08
48	5-Allyloxy-1H-indole-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	376.4	376.06
49	3H-Benzo[e]indole-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	370.3	370.05
50	5-Methyl-2H-pyrazole-3-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	285.2	285.03
51	1-Acetyl-piperidine-4-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	330.3	330.08
52	Cyclohex-1-enecarboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	285.2	285.06
53	3-(2-Methoxy-ethoxy)-thiophene-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	361.3	361.02
54	1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	391.4	391.07
55	7-Methoxy-benzofuran-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	351.2	351.03
56	7-Methoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	367.3	367.01
57	2-Phenyl-2H-[1,2,3]triazole-4-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	348.3	348.04
58	5-Phenyl-thiazole-4-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	364.3	364.01

Number	MolName	Work-up procedure	MW found [M+H]	Exact MW [M+H] calc'd
59	2-Chloro-thiazole-4-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	322.1	321.94
60	2-Benzyl-5-methyl-2H-pyrazole-3-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	375.3	375.08
61	5-Methyl-1-phenyl-1H-pyrazole-3-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	361.4	361.06
62	5-Methyl-1-phenyl-1H-[1,2,3]triazole-4-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	362.5	362.06
63	5-Methyl-2-phenyl-oxazole-4-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	362.4	362.05
64	1H-Indole-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	320.4	320.04
65	4-Acetyl-3,5-dimethyl-1H-pyrrole-2-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	340.3	340.06
66	6-Oxo-1,4,5,6-tetrahydro-pyridazine-3-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	301.3	301.03
67	Thiazole-4-carboxylic acid (2-amino-4,5-dichloro-phenyl)-amide	B	288.3	287.98
68	Benzofuran-2-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	254.4	254.09
69	5-Phenethyl-4,5-dihydro-isoxazole-3-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	311.5	311.15
70	5-Allyloxy-1H-indole-2-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	309.3	309.14
71	Cyclohex-1-enecarboxylic acid (2-amino-pyridin-3-yl)-amide	B	218.4	218.13
72	1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	324.3	324.15
73	7-Methoxy-benzofuran-2-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	284.4	284.10
74	7-Methoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	300.3	300.08

Number	MolName	Work-up procedure	MW found [M+H]	Exact MW [M+H] calc'd
75	2-Phenyl-2H-[1,2,3]triazole-4-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	281.4	281.12
76	5-Phenyl-thiazole-4-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	297.3	297.08
77	2-Benzyl-5-methyl-2H-pyrazole-3-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	308.5	308.15
78	5-Methyl-1-phenyl-1H-[1,2,3]triazole-4-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	295.4	295.13
79	5-Methyl-2-phenyl-oxazole-4-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	295.4	295.12
80	2,5-Dimethyl-4-nitro-2H-pyrazole-3-carboxylic acid (2-amino-pyridin-3-yl)-amide	B	277.3	277.11
81	Benzofuran-2-carboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	255.2	255.09
82	5-Phenethyl-4,5-dihydro-isoxazole-3-carboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	312.3	312.15
83	Cyclohex-1-enecarboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	219.3	219.12
84	3-(2-Methoxy-ethoxy)-thiophene-2-carboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	295.3	295.09
85	1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	325.4	325.14
86	7-Methoxy-benzofuran-2-carboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	285.3	285.10
87	7-Methoxy-benzo[b]thiophene-2-carboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	301.3	301.08
88	2-Phenyl-2H-[1,2,3]triazole-4-carboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	282.3	282.11
89	5-Phenyl-thiazole-4-carboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	298.3	298.08
90	2-Benzyl-5-methyl-2H-pyrazole-3-carboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	309.3	309.15

Number	MolName	Work-up procedure	MW found [M+H]	Exact MW [M+H] calc'd
91	5-Methyl-1-phenyl-1H-[1,2,3]triazole-4-carboxylic acid (4-amino-pyrimidin-5-yl)-amid	B	296.5	296.13
92	5-Methyl-2-phenyl-oxazole-4-carboxylic acid (4-amino-pyrimidin-5-yl)-amide	B	296.4	296.11
93	5-methoxy-benzofuran-2-carboxylic acid (2-amino-phenyl)-amide	A	283.0	283.11
94	6,7-dimethyl-benzofuran-2-carboxylic acid (2-amino-phenyl)-amide	A	281.0	281.13
95	5-nitro-benzofuran-2-carboxylic acid (2-amino-phenyl)-amide	A	298.0	298.08
96	6-methoxy-benzofuran-2-carboxylic acid (2-amino-phenyl)-amide	A	283.2	283.11
97	6-methoxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	282.2	282.12
98	4-chloro-5-methoxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	316.2	316.09
99	3-methoxy-5-methyl-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	296.2	296.14
100	3-pyrrol-1-yl-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	317.2	317.14
101	7-nitro-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	297.1	297.10
102	5,7-dimethoxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	312.2	312.13
103	5-Methoxy-1*H*-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	282.2	282.12
104	6-Methoxy-4-trifluoromethyl-1*H*-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	350.2	350.11
105	5-Methoxy-1-methyl-3-methylsulfanyl-1*H*-indole-2-carboxylic acid (2-amino-phenyl)-amide	A	342.3	342.13

Example 2

In order to investigate the differentiation-inducing effect of the compounds according to this invention, two assays were used.

A) Assay for inhibition of histone deacetylase (HDAC)

Principle :

HDAC deacetylates lysines in histone H4. A 17 aa peptide with TAMRA at the C-terminus and QSY7 at the N-terminus was used as a substrate. Following deacetylation by HDAC the enzyme Lys C is able to cleave the peptide resulting in disappearence of the quench effect and a high signal. Inhibition of HDAC by compounds results in low signals because Lys C has no substrate for cleaving and the quench effect persists.

Assay :

For dose response curves 10 concentrations were diluted starting at 30 uM. 10 ul compound dilution were put into each well of a 384 well plate. 10 ul HDAC (rec. HDAC-1 purified from HEK 293 cells; activity has to be assessed for each preparation) were added. 10 ul peptide was added (1 uM final concentration). After 90 min incubation at room temperature the reaction was stopped by addition of 20 ul test buffer including 3 ug/ml Lys C and 0.075% SDS. After overnight incubation the fluorescent signal of TAMRA was measured, absorption 544 nm, emission 590 nm by Victor 2.

B) E-cadherin upregulation assay

Assay principle

E-cadherin as an adhesion molecule is important for connection of the cells and integrity of tissues. There are tumor cells where E-cadherin protein is downregulated allowing cells to metastasize. Upregulation of the protein in cells where there is not enough E-cadherin is regarded as a marker for the

differentiation of the tumor cell that means leaving the undifferentiated tumor cell status.

The compounds described are able to upregulate expression of E-cadherin in A549. In parallel the proliferation of the cells is examined. The activity of an E-cadherin upregulator is defined as an increase in E-cadherin expression determined by an ELISA. Taken into account is the cell proliferation which is determined by WST-1 in the same well.

Method

The assay is performed in 384-well format. A549 (ATCC : CCL-185), a human lung carcinoma cell line, shows very low expression of E-cadherin. 5000 cells per well are seeded in culture medium with 10% FCS at day 1. At day 2 compounds in different concentrations starting at 40 uM are added at a final concentration of DMSO of 0.5 %. At day 4 WST-1 reagent is added (see instructions of Roche Molecular Biochemicals) and absorption is measured after 45 min (450 nm / 690 nm).

The medium is aspirated, 100 ul fixation solution is added per well and discarded after 30 min, followed by addition of 100 ul blocking solution. Addition of 20µl/well mAb anti-E-cad (clone 6F9), final conc. 0,3µg/ml for 60 min. Three washing steps with PBS/Tween 100µl/well. Addition of mAb anti-mouse-Ig-Biotin (20µl/well) for 60 min. Three washing steps with PBS/Tween. Addition of Streptavidin-POD (20µl/well) for 60 min. Three washing steps with PBS/Tween. Addition of 50µl ABTS substrate for 5 min and finally addition of 12.5 ul oxalic acid to stop the reaction. Measure absorption at 405 nm.

Calculation of EC50 :

% proliferation of cells is substracted from % E-cadherin expression resulting in Delta. The dose response curve resulting from 10 concentrations is calculated using XLfit. EC50 is the concentration where 50 % of cells show real upregulation of E-cadherin protein expression.

Table 2

Compound No.	E-cadherin upregulation EC50 [μM] (Assay B)	HDAC IC50 [uM] (Assay A)
1-38	5.9	11.05

Example 3

Tablet Formulation (Wet Granulation):

Item	Ingredients	mg/tablet			
1.	Compound of formula I	5	25	100	500
2.	Lactose Anhydrous DTG	125	105	30	150
3.	Sta-Rx 1500	6	6	6	30
4.	Microcrystalline Cellulose	30	30	30	150
5.	Magnesium Stearate	1	1	1	1
	Total	167	167	167	831

Manufacturing Procedure:

1. Mix items 1, 2, 3 and 4 and granulate with purified water.
2. Dry the granules at 50°C.
3. Pass the granules through suitable milling equipment.
4. Add item 5 and mix for three minutes; compress on a suitable press.

Capsule Formulation:

Item	Ingredients	mg/capsule			
1.	Compound of formula I	5	25	100	500
2.	Hydrous Lactose	159	123	148	---
3.	Corn Starch	25	35	40	70
4.	Talc	10	15	10	25
5.	Magnesium Stearate	1	2	2	5
	Total	200	200	300	600

Manufacturing Procedure:

1. Mix items 1, 2 and 3 in a suitable mixer for 30 minutes.
2. Add items 4 and 5 and mix for 3 minutes.
3. Fill into a suitable capsule.

Example 4

Pharmacological Test Report

Method

Female Balb/C nu/nu-mice(n = 12 per group), aged 8-10 weeks, were subcutaneously injected with 5*106 SW620 colon carcinoma cells. On day 6, animals with tumor volumes of about 200 mm3 were randomly assigned to treatment groups. The test compound was administered as a fine suspension in 0.5 % methylcellulose with an application volume of 10 ml/kg based on actual body weights. Oral treatment started on day 8 and continued through to day 27 on a once daily, 7 times per week treatment schedule.

Results

Effect on tumor volumes at the end of the study:

Table 3

	Tumor volume (mm^3)	% inhibition vs. control
Vehicle	2200.0	0
Compound of Ex. 38	1029.0	53

The volume of the tumor was determined from the following equation:

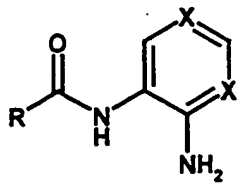
Volume of a tumor = ½ x (major axis) x (minor axis)2

List of References

DE-A 2 062 265
EP-A 0 242 851
EP-A 0 847 992
FR 2 167 954
Hassan, H., et al., Indian J. Chem. 39B (2000) 764-768
Moll, R., et al., Z. Chem. 17 (1977) 133-134
Rastogi, R., and Sharma, S., Indian J. Chem., Sect. B, 21B (5) (1982) 485-487

Patent Claims

1. A pharmaceutical composition comprising, as active ingredient, one or more compounds represented by formula I



(I)

in which

X independently from each other denotes a CH group or a nitrogen atom and

R represents an optionally substituted five or six membered nonaromatic carbocyclic ring or an nonaromatic or aromatic heterocyclic ring, whereby the ring system may also be condensed with a 6-membered, optionally substituted carbocyclic aromatic ring;

and its pharmaceutically acceptable salts

in admixture with pharmaceutically acceptable excipients or diluent,

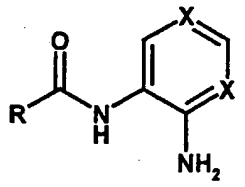
with the proviso that

R does not represent optionally substituted pyridinyl.

2. A pharmaceutical composition according to claim 1 comprising a compound as active ingredient selected from the group consisting of

Benzofuran-2-carboxylic acid (2-amino-phenyl)-amide
5,6-Dimethoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-phenyl)-amide
5-Propoxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide
5-Allyloxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide
7-Methoxy-benzofuran-2-carboxylic acid (2-amino-phenyl)-amide
7-Methoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-phenyl)-amide
Thiazole-4-carboxylic acid (2-amino-phenyl)-amide.

3. A compound of formula I



(I)

in which

X independently from each other denotes a CH group or a nitrogen atom and

R represents an optionally substituted five or six membered nonaromatic carbocyclic ring or an nonaromatic or aromatic heterocyclic ring, whereby the ring system may also be condensed with a 6-membered, optionally substituted carbocyclic aromatic ring;

and its pharmaceutically acceptable salts,

with the proviso that

(a) if X denotes CH, R does not represent
halogenated benzothiophene; halogenated thiazolyl; N-benzyl-2-acetylamino-4,5-dimethylpyrrole-3-yl; optionally substituted 1,4-oxathiine-3-yl; optionally substituted pyridinyl; pyridazine-5-yl which is substituted by one to three substituents selected from methyl, methoxy, methoxycarbonyl or carboxyl;

or

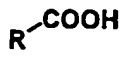
(b) if one or both X in formula (I) denote NH, R does not represent optionally substituted pyridinyl.

4. A compound according to claim 3, whereby the compound is selected from the group consisting of

5,6-Dimethoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-phenyl)-amide
5-Propoxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide
5-Allyloxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide

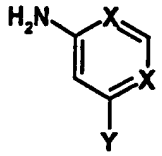
7-Methoxy-benzofuran-2-carboxylic acid (2-amino-phenyl)-amide
7-Methoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-phenyl)-amide.

5. Process of manufacturing a compound according to claim 3 by reacting a carboxylic acid of formula II



II

in which R has the meaning according to claim 3,
with a compound of formula III



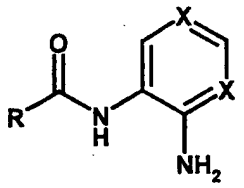
III

in which

Y represents an amino or a protected amino group and X represents CH or N and if X denotes CH, the six-membered ring may be substituted by one or two halogen atoms;

in the presence of activation reagents,
whereafter the protection group is cleaved, if any, to form the amino group and the compounds of formula I are converted, if desired, into its pharmaceutically acceptable salts.

6. Use of one or more compounds of formula I



(I)

in which

X independently from each other denotes a CH group or a nitrogen atom and

R represents an optionally substituted five or six membered nonaromatic carbocyclic ring or an nonaromatic or aromatic heterocyclic ring, whereby the ring system may also be condensed with a 6-membered, optionally substituted carbocyclic aromatic ring;

and its pharmaceutically acceptable salts;

with the proviso, that

R does not represent optionally substituted pyridinyl,

for the preparation of medicaments for the treatment of cancer.

7. Use of one or more compounds according to claim 6, whereby the compound is selected from the group consisting of

Benzofuran-2-carboxylic acid (2-amino-phenyl)-amide
5,6-Dimethoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-phenyl)-amide
5-Propoxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide
5-Allyloxy-1H-indole-2-carboxylic acid (2-amino-phenyl)-amide
7-Methoxy-benzofuran-2-carboxylic acid (2-amino-phenyl)-amide
7-Methoxy-benzo[b]thiophene-2-carboxylic acid (2-amino-phenyl)-amide
Thiazole-4-carboxylic acid (2-amino-phenyl)-amide.

8. Use of one or more compounds according to one of the claims 3 or 4, or a pharmaceutical composition according to claim 1 or 2, as histone acetylation-inducing agents for the treatment of cancer.

9. A method for the treatment of cancer, characterized by administring to a patient in need of such treatment an effective amount of one or more compounds of one of claims 3 or 4, or a pharmaceutical composition according to claim 1 or 2.

10. A method for inhibiting tumor cell proliferation, characterized by induction of histone acetylation in a tumor cell, due to administring to said tumor cell an effective amount of one or more compounds according to one of the claims 3 or 4, or a pharmaceutical composition according to claim 1 or 2.